

ICSAFS

International Conference on Sustainable Agriculture and Food Security: *Challenges and Opportunities*

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Proceeding

(Oral Papers)

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Inducing Somatic Embryos of Soybean *Glycine max* and *Glycine soja* on Sucrose Concentrations Variation

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Abstract

Production and productivity of Soybean in Indonesia have not met a national demand. The government program is to improve Soybean productivity and production to achieve self sufficiency on 2015. Genotype and sucrose in the medium tissue culture is important inducing somatic embryos. The objectives of this experiment was to inducing somatic embryos of *Glycine max* varieties Anjasmoro and *Glycine soja* varieties Mallika at the variation of sucrose concentration for MS medium by *in vitro*. The research was used immature cotyledons explant which was conducted at Greenhouse and Biotechnology Laboratory, Agrotechnology of UPN "Veteran" Yogyakarta, Indonesia from Mei to October 2010. The experiments were arranged in factorial Completely Randomized Design with two factors and replicated ten times. The first factor was explants soybeans were: Mallika (*Glycine soja*) and Anjasmoro (*Glycine max*). The second factor was concentration sucrose: 20 g/l, 30 g/l, and 40 g/l. Data were subjected to an analysis of variance followed by Dunnet's Significance Test (DST) at 5% significance level. The results showed that material explant *Glycine max* and *Glycine soja* not induced somatic embryos. The best sucrose concentrations 20 g/l for MS medium increased to time of embryos (days), growth percentage of embryos and fresh weight callus. The combination treatment *Glycine max* and sucrose 20 g/l to increased dry weight callus.

Keyword: Embryos somatic, soybean, sucrose

Introduction

The Soybean is a crop that has rich nutritional and includes 10 crop commodities besides rice and corn commodities. In recent years, soybean production is still the range of 600-700 thousand tons per year, while the demand has reached 2.0 million tons. The low national production of soybean, as well as the total area of plantations is still limited or declining, as well as productivity per unit area remains low. This is caused by the use of low-quality seeds and by the onset of the disease (Anonymous, 2010).

Propagation in the conventional soybean plants generally requires a long time as well as a vast place so its needs to be done in biotechnology and by vegetative propagation of plants through tissue culture techniques (*in vitro*). Technology is an *in vitro* culture technique in plant breeding pieces of tissue in a sterile artificial media. The technology is based on the properties of the cell that each individual is able to form a new whole that has properties identical to the parent cell, especially the young (Wahyurini, 2008). The medium used for cultivating the tissue sections containing foods such as macro elements and micro nutrients. In addition, in the medium was also added source of carbon derived from sucrose, vitamins and growth regulators that serve to spur growth and improve the ability of cells to multiply and develop into a candidate plant (Gamborg and Shyluk, 1981 and George and Sherington, 1984).

Regeneration of plant tissue culture can be done through somatic embryogenesis and organogenesis. Somatic embryogenesis is widely used because it can accelerate the discovery of the success of transgenic crops with a high opportunity for transformation of somatic embryos which can be derived from a somatic cell. Somatic embryos can be induced directly from tissue explants or indirectly through a callus phase. Plant regeneration from callus cultures often show genetic diversity that somatic embryogenesis is more efficiently used directly in the application of biotechnology for plant breeders.

Currently somatic embryogenesis is well known as regeneration induction to way of tissue culture explants, or indirectly through a callus phase. Its success is largely determined by media formulations optimized for each stage of culture (Yusnita, 2003). The successful regeneration of soybean plants is also highly dependent on the genotype used. From previous studies induction of somatic embryogenesis in peanut, which mostly done by using several concentrations of sucrose, still produce a diverse number of embryos. So, on that ground, conducted research on somatic embryos soybean of white and black, is still the same family with peanuts as *Leguminosae*. The research to know about the concentration of sucrose is right for the formation of soybean embryos. A problem in this study is on how the provision concentration sucrose effect on increasing the amount of soybean embryos and in a short time of planting material a bit.

Materials and Methods

This research has been conducted in the greenhouse and laboratory Agrotechnology Department of Biotechnology in May until October 2010. The materials used were: soybean seed varieties *Anjasmoro* and *Mallika*, poly bags, sand, manure, MS medium (Murashige and Skoog), jelly, sucrose, 2.4 D, disinfectants (Furadan, agrimycin, Benlate, 96% alcohol, bayclin 50%, sublimate 0.1%), sterile distilled water, aluminum foil, filter paper, gloves, and detergents. The tools used were: the culture bottles, beakers, Petridis, pH sticks, Laminar Air Flow (LAF), disinfect sets, lighting Bunsen and autoclave. The experiment was conducted using a Factorial Completely Randomized Design with 2 factors, with ten replications.

The First factor was explants material (genotype soybean) comprising 2 levels: white soybean Anjasmoro (K1) and black soybean Mallika (K2). Whereas the second factor was concentration of sucrose, which comprises three levels: 20 g / l (S1), 30 g / l (S2) and 40 g / l (S3). The data were analyzed by Variance Analysis at the level 5%. Therefore, to know there were a significant differences between the treatments then the test by *Dunnet's Significance Test (DST)* at 5% significance level.

Results and Discussion

The results of the present analysis shows that the treatment appears embryo explants material significantly affect the concentration of sucrose but the treatment did not significantly affect time of embryos. Average value the time of embryos can be seen in Table 1.

Table 1 shows that treatment of explants s K1 faster as the time of embryo than K2 treatment. At treatment concentrations of sucrose showed a significant S1 faster when compared treatments S2 and S3. In the early growth response of black soybean callus showed a faster growth than white soybean. In morphology the size and shape of white

soybeans *Anjasmoro* greater than *Mallika* black soybeans, so cotyledon as food reserves could supply the cells forming the meristem cells.

Table 1. The mean time of embryos (days)

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	Mean
K1 (white soybean)	22,25	22,25	24,75	23,167 a
K2 (black soybean)	25,50	26,25	26,25	26,00 b
Mean	23,88 p	24,38 q	25,25 q	(-)

Note : Mean followed by same small letter indicates no significant difference in the test DST 5%. Sign (-) showed no interaction

The embryo appears at day 22 after planting some sucrose treatment. Provision of sucrose with a concentration of 20 g / l markedly more rapid time of the embryo, this is due to sucrose with 20 g / l which was the best carbon source that acts as a raw material producing energy in the process of respiration (Katuuk, 1984). This energy which is used in cell-cell division to form embryos. The results of the analysis of the growth percentage of embryos showed that the treatment material did not significantly, but the concentration of sucrose significantly. Average value of the growth percentage of embryos can be seen in Table 2.

Table 2. The mean of the growth percentage of embryos

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	Mean
K1 (white soybean)	81,25	75,00	81,25	79,17 a
K2 (black soybean)	87,50	57,50	68,75	71,25 a
Mean	84,38 p	66,25 r	75,00 q	(-)

Note book: Mean followed by same small letter indicates no significant difference in the test DST 5%. Sign (-) showed no interaction

Table 2 shows that the treatment was not significantly different explants K1 with K2 treatment. At treatment concentrations of sucrose showed a greater percentage of significant S1 explants which are capable of forming embryos than treatment S2, and S2 greater than S3 significant. There was no interaction between sucrose concentration and explants material percentage of explants capable of forming embryos.

Treatment of different materials the genotype explants showed no significant difference in the percentage of embryos. Soybean embryo explants of black and white soybeans has the ability and equal opportunity for the proliferation or growth of cells so that the percentage of growing embryo no significant difference. The key to success in the formation of callus tissue of life is the existence of a sterile nutrient medium that has an optimum and suitable environment and culture (Ursila, 2004).

Provision of sucrose with a concentration of 20 g / l was the significant percentage of the growth of embryos than other treatments. The success of plant tissue culture is highly dependent on the media used. Tissue culture media does not only provide macro and micro nutrients but also the carbohydrates that in general the form of sugar. Sugar is a source of carbon instead of carbon usually obtained plants from the atmosphere in the form of CO2 into a component for photosynthesis (Gunawan, 1988).

The results of the analysis of the number of embryos per explants showed that the treatment material and the concentration of sucrose does not significantly affect the number of embryos per explants The average number of embryos per explants can be seen in Table 3.

Table 3 shows that treatment of explants K1 and K2 were not significantly different. At the concentration of sucrose treatment S1, S2 and S3 were not significantly different between treatments. There was no interaction between sucrose concentration and explants material to the average number of embryos per explants.

Table 3. Average number of embryos per explants

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	mean
K1 (white soybean)	31,39	29,67	32,21	31,09 a
K2 (black soybean)	27,29	27,75	37,34	30,79 a
mean	29,34 p	28,71 p	34,78 p	(-)

Note book: Mean followed by same small letter indicates no significant difference in the test DST 5%. Sign (-) showed no interaction

Treatment of different genotype materials explants showed no significant difference in the number of embryos per explants. In forming callus growth, white soy faster growth, but the subsequent development of both materials explants showed the same ability to form embryos. This is due to the development of cells forming embryonic cells is influenced by the nutrients contained in the media.

Provision of sucrose with a concentration of 20 g / l apparent greater number of embryos per explants compared to other treatments. This is because sucrose was an important carbon source used as a constituent of cells, cell division, cell enlargement and differentiation of cells that can form the plant shoots, and embryos as well (George and Sherrington, 1984). The results of the analysis of wet weight of explants material shows that the treatment effect was not significant, but the treatment concentration of sucrose significantly affects callus wet weight. Average value of wet weight of callus can be seen in Table 4.

Table 4. shows that treatment of explants K1 and K2 were not significantly different in the concentration of sucrose treatment showed S1, the callus markedly more severe than the S2 and S3. There was no interaction/relation between sucrose concentration and explants material to wet weight of callus.

Treatment of different genotype materials explants showed no significant difference in wet weight of callus. In forming callus growth, white soybean faster growth, but subsequent

developments showed the same ability proliferating. This is due to the development of callus formation and embryos are fixed, so the plant will produce the same wet weight (Andarwening, 2009).

Table 4. The mean wet weight of callus (g)

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	Mean
K1 (white soybean)	1,365	1,142	1,212	1,240 a
K2 (black soybean)	1,281	1,098	1,177	1,186 a
Mean	1,323 p	1,120 q	1,195 q	(-)

Note book: Mean followed by same small letter indicates no significant difference in the test DST 5%. Sign (-) showed no interaction

On a wet weight parameters of callus that the granting of the concentration of sucrose 20 g/l showed the greatest callus wet weight compared with other treatments. The state thus induced cells in tissue explants grown on media with the addition of sucrose 20 g/l more rapidly receive the nutrients necessary for its development, while also influenced by the ability of the plant itself in receiving nutrients. Growth in the general sense is the formation, among others, the volume size, weight and number of cells (Salisbury and Ross, 1992). Dry weight analysis results showed that treatment of explants material and the concentration of sucrose does not significantly affect the dry weight of callus. There was interaction between the materials treated explants with sucrose concentration on dry weight of callus. Average value of dry weight of callus can be seen in Table 5.

Table 5. The mean dry weight of callus (g)

Treatment	S1 (20 g/l)	S2 (30 g/l)	S3 (40 g/l)	mean
K1 (white soybean)	1,734 ab	1,722 b	1,769 a	1,742
K2 (black soybean)	1,699 b	1,530 c	1,701 b	1,643
mean	1,717	1,626	1,735	(+)

Note book: Mean followed by same small letter indicates no significant difference in the test DST 5%. Sign (-) showed no interaction

Table 5 shows that the combination treatment K1S3 (white soybean and sucrose 40 g/l) did not differ significantly with treatment K1S1 (white soybean and sucrose 20 g/l) but significantly different from the other treatment combinations on the dry weight of callus. Combination treatment K2S2 (black soybeans and sucrose 30 g /l) were significantly lighter weight than other treatments. According to Septiana (2010) the process of explants growth and development can be realized by the assimilated accumulation which would be translocation into various cell explants required. If explants are not able to form assimilate sufficiently, the explants will growth vary.

K1S3 combined treatment (white soybean and sucrose 40 g / l) and K1S1 (white soybean and sucrose 20 g / l) produced the greatest dry weight of callus compared to other treatments. Dry weight of callus showed progression and cell growth. The white soybeans in sucrose concentration of 20 or 40 g / l produced the greatest dry weight of callus may be due to the density concentration and induces the cells to maintain the acidity of H + so that the water potential in the cell down and eventually enters the cell and places cell development. Provision of sucrose as a substitute for carbon is very involved in the process of photosynthesis, so the number of assimilate formed in the formation of many organs of plants (George and Sherrington, 1984).

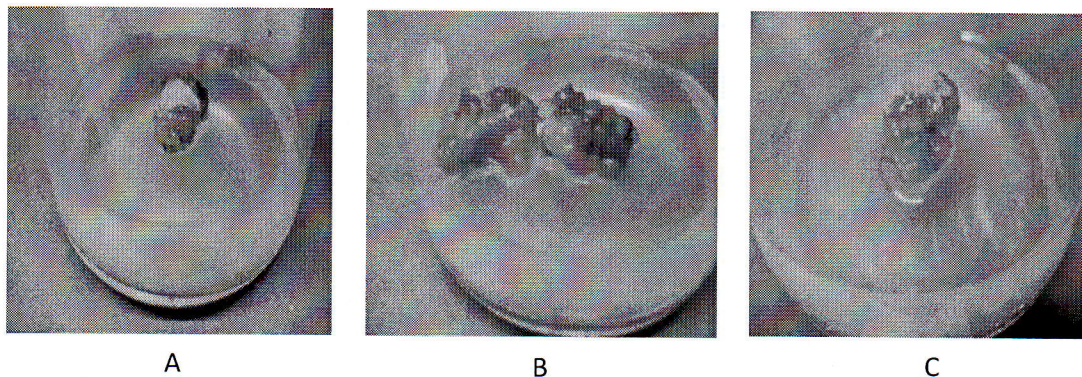


Figure 1. Embryo somatic with treatment K1S1 (A) after three weeks ; K2S1 (B) after four weeks ; K1S1 (C) after ten weeks

Conclusion

1. Treatment of white soybean (K1) and black soybeans (K2) does not affect the induction of somatic embryos *in vitro*.
2. The treatment concentration of sucrose 20 g / l may increase to time of embryos (days), growth percentage of embryos and fresh weight callus.
3. The combination treatment *Glycine max* and sucrose 20 g/l to increased dry weight callus.

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